Platform: Calcium Fluxes, Sparks, & Waves

1849-Plat
EC Coupling Latency in Rat Ventricular Myocytes
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Rapid activation of sarcolemmal L-type Ca channels (LCCs), Ca influx and activation of Ca-induced Ca release (CICR) from the sarcoplasmic reticulum (SR) are crucial to cardiac excitation-contraction (EC) coupling. The uniformity and duration of a Ca transient elicited by an action potential (AP) depends on LCC activation kinetics and ability of the consequent Ca influx to trigger Ca sparks. In contrast to some earlier work, recent studies have suggested that EC coupling 'fidelity' is extremely low and that as many as 20-30 LCC openings are required to trigger a Ca spark. This result would suggest that the latency to Ca spark activation is dominated by LCC activation, (although this has not been shown). To investigate this idea, step voltage-clamp protocols were used to activate Ca transients and Ca sparks. In one series of experiments, LCCs were first activated by a pre-pulse. The latency of SR Ca release decreased with increasing depolarizing step potential, in qualitative agreement with the expected voltage-dependence of LCC activation. In contrast, the latency of SR Ca release increased with increasing step potential after a strong pre-pulse which should have removed the latency for LCC activation. The difference between these results reflects the influence of LCC gating kinetics on EC coupling latency. The results for Ca spark and Ca transient latency suggest that only a few LCC openings are required to trigger a Ca spark and that during a Ca transient, LCC availability was ~3-fold larger. To test this interpretation, a computer model was constructed to simulate stochastic LCC openings, Ca appearance inside the dyad and Ca-dependent gating of ryanodine receptors. The model shows that the recorded Ca spark latencies can be reproduced when the number of LCCs that initiate SR Ca release is 1-2 and 5-6 for Ca transients.

1850-Plat
Termination of Calcium Sparks: An Emergent Property from Stochastic RyR Gating and Dyad Geometry
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Many cells depend on 'calcium-induced calcium release' (CICR), an inherently regenerative process due to the Ca^{2+}-dependent gating of ryanodine receptors (RyRs) in the SR. Ca^{2+} sparks, as seen in muscle, reflect the concerted gating of groups of RyRs in specialized signalling domains in the junctions between the SR and surface membrane. However, the mechanism(s) responsible for the termination of regenerative CICR during the evolution of Ca^{2+} sparks remain uncertain. Rat cardiac RyR gating was recorded at physiological Ca^{2+}, Mg^{2+} and ATP levels and incorporated into a 3D spatial model of the cardiac dyad which reproduced the time-course of Ca^{2+} sparks, Ca^{2+} blinks and Ca^{2+} spark restitution. Model CICR termination was robust, relatively insensitive to the number dyadic RyRs and automatic. This emergent behavior arose from the rapid development and dissolution of nanoscopic Ca^{2+} gradients within the dyad. These simulations show that CICR does not require intrinsic inactivation mechanisms for stability and cessation of regeneration arises from local control at the molecular scale via a process we call 'induction decay'.

1851-Plat
Direct CaMKII Activation by Endogenous Nitric Oxide Modulates Calcium Spark Frequency in Cardiomyocytes
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During β-adrenergic stimulation of cardiomyocytes, phosphorylation of the ryanodine receptors (RyRs) by protein kinases may contribute their increased open probability. We previously reported that β-adrenergic stimulation (β-ARS) with isoproterenol (Iso) increases the Ca^{2+} spark frequency by about 4-fold in quiescent, voltage-clamped cardiomyocytes. Kinase inhibitors (KN-93, AIP, H89) and nitric oxide synthase (NOS) inhibitors (L-NAME, AAAN) indicated that CaMKII and NOS were involved in the change of spark frequency.
Here we show a new mechanism where CaMKII is activated by nitric oxide (NO). using confocal NO imaging with DAF-2 we observed a significant (~19%) raise in endogenous NO production matching the time-course of the increase in spark frequency upon β-ARS. In contrast, this increase in NO was not observed in the presence of L-NAME, confirming that the DAF-2 fluorescence was reflecting NO and not ROS. In parallel experiments, bypassing the classical β-ARS pathway by application of a NO donor, increased the frequency of sparks to the same extent as during β-ARS with Iso. This increase induced by NO was blocked by the CaMKII inhibitor AIP, suggesting that NO first activated CaMKII, which subsequently phosphorylated the RyRs. Biochemical in-vitro CaMKII activity assays confirmed direct activation by NO, in a Ca^{2+} independent manner. CaMKII has been reported to be activated by ROS. However, control experiments revealed that ROS were not involved in the examined pathway. Neither incubation of the cells with SOD mimetics or ROS scavengers (Mn-TBAP, TIRON) prevented the increase in spark frequency upon Iso application.
Based on these findings we conclude that endogenously produced NO activates CaMKII, contributing to the regulation of RyR Ca^{2+} sensitivity and Ca^{2+} spark frequency upon β-adrenergic stimulation of cardiomyocytes. Supported by SNF

1852-Plat
ROS and Mitochondrial Membrane Potential Dependent Modulation of Calcium Signaling in the Heart
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Experimental measurements suggest that large mitochondrial Ca^{2+} fluxes are normal and physiological. Here a quantitative investigation of Ca^{2+} movement through the inner mitochondrial membrane (IMM) has been conducted in isolated rat cardiomyocytes. In these cells, elevated local $[Ca^{2+}]_i$ (10 μM) bathes the end microdomains of the thousands of intermyofibrillar mitochondria with every heartbeat. Moreover, the mitochondrial inner membrane potential ($\Delta\Psi_{mito}$) of ~ −180 mV provides a strong electrochemical gradient for the movement of Ca^{2+} from the cytosol into the matrix. The role of $\Delta\Psi_{mito}$ in Ca^{2+} uptake by mitochondria was tested by examining Ca^{2+} sparks and $[Ca^{2+}]_i$ transients in cells or regions of cells where $\Delta\Psi_{mito}$ is dissipated by photon stress. In addition to the changes in electrochemical potential for Ca^{2+} entry, depolarization of mitochondria was associated with an increase in cellular reactive oxygen species (ROS) as measured by DCF. Consistent with recent experimental findings, we report a role for localized ROS in altering Ca^{2+} signaling within the depolarized region. Furthermore, using novel quantitative analysis, we separate the influence of ROS and $\Delta\Psi_{mito}$ on the observed changes in Ca^{2+} signaling. In doing so, new insights into mitochondria contribution to cytosolic calcium handling are obtained.

1853-Plat
CaMKII-Dependent Phosphorylation Modulates Ca^{2+} Cycling in Sinoatrial Node Cells to Regulate Cardiac Pacemaker Function
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Spontaneous beating of rabbit sinoatrial node cells (SANC) is linked to rhythmic, submembrane, sarcoplasmic reticulum (SR) generated local Ca^{2+}releases (LCR), which activate inward Na^{+}/Ca^{2+}exchange current to impart exponential increase of the late diastolic depolarization rate and fire an action potential (AP). Rabbit SANC have high basal PKA-dependent phosphorylation, but basal CaMKII-dependent phosphorylation has never been ascertained. Here we show that SANC maintain high basal PLB phosphorylation at Thr^{17} site (western blotting), employed as a marker of CaMKII-dependent protein phosphorylation, that is markedly suppressed by CaMKII inhibitor, KN-93, but not its inactive analog KN-92. Considering that CaMKII activity is critically dependent on intracellular $[Ca^{2+}]_i$ we employed BAPTA-AM to chelate $[Ca^{2+}]_i$. Both KN-93 and BAPTA-AM markedly decrease the LCR size (confocal line-scan imaging), LCR number per each spontaneous cycle and prolong the LCR period (time between AP-induced Ca^{2+} transient and subsequent LCR). The increase in the LCR period predicts concurrent increase in the spontaneous cycle length. To delineate mechanisms of CaMKII-dependent regulation of Ca^{2+} cycling, avoiding interference of L-type Ca^{2+}channels, which are also regulated by CaMKII, we permeabilized SANC with saponin. Elevation of cytosolic $[Ca^{2+}]$ from 50 to 150 nmol/L in permeabilized SANC significantly increases the LCR size and amplitude due, in part, to a marked increase of PLB phosphorylation at Thr^{17}site and resultant increase in the SR Ca^{2+} content and, in part, to RyR phosphorylation at PKA- and CaMKII-dependent Ser^{2809} site (immunostaining). CaMKII-inhibitor